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(71) Applicant: THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK [US/US]; West 116th Street and Broadway, New York, NY 10027 (US).

(72) Inventors: CANFIELD, Robert, E. ; 601 West 113th Street, New York, NY 10025 (US). IMAMURA, Sadomi ; 2-17, Oura-Machi, Nagasaki City 850 (JP). ARMSTRONG, Elmo, G. ; 5093 Post Road, Bronx, NY 10471 (US). BIRKEN, Steven ; 120 Oneida Avenue, Dumont, NJ 07628 (US). COLE, Laurence, A. ; 3244 Bolgos Circle, Ann Arbor, MI 48105 (US).

(74) Agent: WHITE, John, P.; Cooper, Dunham, Griffin & Moran, 30 Rockefeller Plaza, New York, NY 10112 (US).

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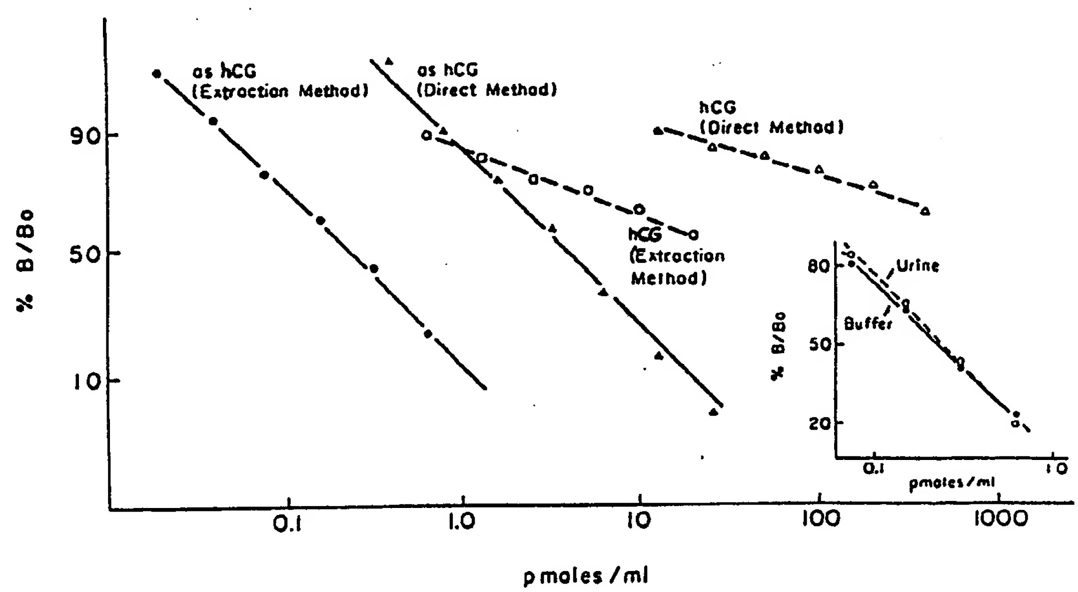


(57) Abstract

A method for determining the presence of soluble desialylated glycoproteins in biological fluids. The method comprises contacting a sample of the biological fluid with a suitable amount of an appropriate lectin capable of selectively binding to the desialylated glycoprotein to produce a complex. The resulting complex is separately recovered from the biological fluid. The recovered complex is contacted under appropriate conditions with at least one detectable antibody directed to an antigenic determinant on the desialylated glycoprotein and capable of selectively binding to glycoprotein present in the complex. The presence of antibodies so bound is detected and, thereby, the presence of desialylated glycoprotein in the biological fluid determined.

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# LECTIN - ANTIBODY SANDWICH ASSAY FOR DESIALYLATED GLYCOPROTEINS

## Background of the Invention

The invention described herein was made with Government support under grant numbers HD15454-03 and RR00645-13 from the National Institute of Child and Health Development, United States Department of Health and Human Services. The Government has certain rights in the invention.

Within this application several publications are referenced by arabic numerals within parentheses. Full citations for these references may be found at the end of the specification immediately preceding the claims. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

Human chorionic gonadotropin (hCG) purified from urine of pregnant women has been shown to have approximately 30% carbohydrate content, consisting of two N-asparagine linked carbohydrate side chains on each of its and subunits (1) and four O-serine linked side chains attached to the COOH-terminal peptide region of its subunit (2,3). In 1981 Nishimura et al. (4) observed that hCG purified from the urine of a patient with choriocarcinoma had a reduced carbohydrate content and that this difference was due to a low sialic acid content. Later studies of the hCG from th same pati nt (5) r v al d unusual N-asparagine linked oligosaccharide side chains of which 97% were free of sialic acid. Studi s by Amr et al. (6,7) have

indicated that the porportion of total hCG which is desialylated in the urine of women with choriocarcinoma is somewhat variable but is consistently higher than that from pregnancy urine. The latter studies also indicated that in addition to desialylated hCG, a small molecule with properties similar to those of the free desialylated hCG COOH-terminal peptide (asβCTP) was present in the urine of women with choriocarcinoma but not in pregnancy urine. Those findings suggested that excretion of desialylated forms of hCG might be characteristic of patients with choriocarcinoma and measurement of these forms might be valuable in diagnosis and management of the disease.

Highly specific antisera to asβCTP have been developed (8) and applied to the detection of desialylated forms of hCG in clinical situations (6,7). However, only moderate sensitivity can be obtained in RIA systems using these antisera. In addition, analyses of urine specimens using these antisera have previously relied upon sample preparation by gel filtration chromatography (6,7). Clinical studies of the production of desialylated forms of hCG by patients with trophoblastic neoplasia would be facilitated by the availability of simple yet highly sensitive and specific methods for the detection of ashCG in urine.

An alternate approach to the detection of glycoproteins is to utilize lectins. These carbohydrate binding proteins, have proved to be useful reagents for probing structural f atur s f cell surface glycoproteins and for is lating glycopr t ins. In the pres nt study we have exploited th carb hydrate binding properties of tw l ctins, peanut lectin, (<u>Arachis hypogea</u> agglutinin, PNA), and castor bean lectin, (<u>Ricinus</u>

*communis* agglutinin, RCA), to develop highly specific methods for the detection of ashCG in urine. PNA and RCA specifically bind the desialylated O-serine (9,10) and N-asparagine (9) linked oligosaccharides of hCG respectively. Solid phase lectin is utilized to extract ashCG from urine, and lectin bound ashCG is then measured utilizing a purified and radiolabeled monoclonal antibody or rabbit antiserum. The form of desialylated hCG detected is dependent not only on the carbohydrate specificity of the lectin but the peptide specificity of the monoclonal antibody or antiserum employed. This unique type of assay has been described as a Lectin-Immunoradiometric Assay (LIRMA) and may be extended to the measurement of a variety of soluble glycoproteins by using combinations of lectins and antibodies with different specifications.

Lectins are proteins or glycoproteins of non-immune origin long known to be useful for agglutinating erythrocytes and other types of cells and useful for studying cell surface properties. However, as macromolecules it might be unexpected that the binding of a lectin to a glycoprotein, particularly a relatively small glycoprotein such as hCG, would prevent subsequent or contemporaneous binding of an antibody directed to an antigenic determinant on the glycoprotein. Unexpectedly, it has been found that the combined use of a lectin which selectively binds to a specific sugar moiety and of an antibody provides a highly sensitive and specific method for qualitatively detecting or quantitatively determining a desialylated glyc protein.

Specifically the method provid d permits xploitati n of th dual natur of glycoproteins. Th l ctin

component binds specifically to the carbohydrate moiety while the antibody component binds specifically to the peptide moiety. As a result, one may distinguish glycoproteins on the basis of a carbohydrate moiety which may be critical if the peptide regions are identical. An example of the importance of the invention is the ability to distinguish desialylated hCG from sialylated hCG and, thereby, diagnose gestational trophoblastic disease.

## Summary of the Invention

A method for determining the presence of a soluble desialylated glycoprotein such as human chorionic gonadotropin in a biological fluid such as urine or blood is provided. This method comprises contacting a sample of the biological fluid with a suitable amount of an appropriate lectin capable of selectively binding to the desialylated glycoprotein to produce a complex. Examples of such lectins include peanut lectin and castor bean lectin. The resulting complex is separately recovered from the biological fluid. The recovered complex is contacted under appropriate conditions with at least one detectable antibody such as radiolabeled or fluorescently labeled monoclonal antibody directed to an antigenic determinant on the desialylated glycoprotein and capable of selectively binding to glycoprotein present in the complex. The presence of antibody so bound is detected and, thereby, the presence of desialylated glycoprotein in the biological fluid determined.

A method for quantitatively determining a soluble desialylated glycoprotein in a biological fluid is also provided. This method comprises contacting a sample of the biological fluid with a suitable amount of an appropriate lectin capable of selectively binding to the desialylated glycoprotein to produce a complex. The resulting complex is separately recovered from the biological fluid. The complex so recovered is contacted under appropriate conditions with a pr determin d amount of at least one det ctable antibody directed to an antigenic determinant on th d sialylat d glycoprot in and capabl of selectively binding to glycoprotein present in th complex. The

amount of antibody so bound is determined and, thereby, the amount of desialylated glycoprotein in the biological fluid determined.

Thus, this invention provides both qualtitative methods for detecting and quantitative methods for determining the presence in biological fluids of soluble glycoproteins. As will be clear to those skilled in the art to which this invention relates, these methods may be readily modified in several respects. Thus, the lectin rather than the antibody may be detectably labeled; the antibody rather than the lectin may be employed to initially contact the sample or the sample may be simultaneously contacted with the antibody and the lectin.

## Brief Description of the Figures

Figure 1: R141 RIA of ashCG with extraction method (●----●) and direct method (▲----▲), and hCG with extraction method (o---o) and direct method (△---△). The extraction method uses a solid phase coupled monoclonal antibody to hCG β subunit for extraction of hCG or ashCG from buffer or urine.

Figure on lower right corner: R141 RIA (extraction method) of ashCG from buffer (o----o) and normal male urine (o----o).

Figure 2: Characteristics of four lectin immunoradiometric assays (LIRMA) for measurement of ashCG in buffer or urine. Comparisons of dose responses were made for the following hormones or fragments: AshCG (o----o), ashCGβ (o----o), asβ-CTP (□----□), hCG (▲----▲), hCGβ (△----△) and hLH (■----■). The LIRMAs illustrated are as follows: A: PNA-$^{125}$I-R525, B: PNA-$^{125}$I-B105, C: PNA-$^{125}$I-B107, D: RCA-$^{125}$I-R525.

Inserts on upper right corners of panels A and D illustrate measurement of ashCG in buffer (O----O) and normal male urine (O----O) using PNA-$^{125}$-R525 (A) and RCA-$^{125}$-I R525 (D).

Figure 3: Sephadex G-100 gel filtration of a urine concentrate of a patient with choriocarcinoma (panel A) and of a normal pregnant woman (panel B). 10 ml of the urine c ncentrat was applied to a column (2.5 x 196 cm) of Sephadex G-100, and elut d with 0.05 M Tris-HCl buffer containing 0.1 M NaCl (pH 7.4). Fractions f 6 ml w r coll ct d and aliquots were assayed in various

assay systems. Total hCG was determined by R529 RIA (●), and ashCG by R141 RIA (direct method) (o) and PNA-$^{125}$I-R525 (▲) LIRMA. 100,000 cpm of $^{125}$I-hCG in 10 ml of the same buffer was eluted in separate run as a marker. The insert in panel B illustrates the amounts of asialo hCG immunoreactivity detected using the three methods on an expanded scale.

## Detailed Description of the Invention

A method is provided for determining the presence of a soluble desialylated glycoprotein in a biological fluid which comprises contacting a sample of the biological fluid with a suitable amount of an appropriate lectin capable of selectively binding to the desialylated glycoprotein to produce a complex. The resulting complex is separately recovered from the biological fluid. The so recovered complex is contacted under appropriate conditions with at least one detectable antibody directed to an antigenic determinant on the desialylated glycoprotein and capable of selectively binding to glycoprotein present in the complex. The presence of antibody so bound is detected and, thereby, the presence of desialylated glycoprotein in the biological fluid is determined.

The soluble desialylated glycoprotein may be hCG, thyroglobulin (11), carcinoembryonic antigen or CA19-9 (12), or any other desialylated glycoprotein or subunit thereof, e.g. desialylated hCG.

The biological fluid may be urine, blood, semen, saliva, pus or any other biological fluid, a sample of which one wishes to examine. Typcially, biological fluid will be obtained from a subject, e.g. a human patient. Thus, for example, desialylated hCG may be detected in human urine.

A sample of the biological fluid is obtain d and c ntact d with a suitabl amount of an appr priate l ctin to produce a compl x. Th c ntact typically involves simply adding th l ctin to the sample und r ambient conditions. The amount and type f l ctin

added may vary widely depending upon factors well known in the art such as the concentration of soluble glycoprotein normally present in the sample and the nature of the glycoprotein. Typcially, the amount of lectin added will be in molar excess of the amount of glycoprotein present in the sample.

Appropriate lectins are lectins capable of selectively binding to the desialylated glycoprotein. Numerous lectins are known which selectively bind to specific sugar moieties and which may therefor be employed in this invention. For example, peanut lectin may be derived from Arachis hypogea. Such lectin selectively binds to the carbohydrate structure Galβ1⟶3GalNAc. Another example is castor bean lectin derived from Ricinus communis which selectively binds to the carbohydrate structure Galβ1⟶4GlcNAc.

The complex which results from contacting the sample of biological fluid with the lectin is then separated from the biological fluid, e.g. by centrifugation followed by decantation of the supernatant. However, other methods of recovery may be employed such as filtration.

The separately recovered complex is contacted with at least one detectable antibody directed to an antigenic determinant on the desialylated glycoprotein and capable of selectively binding to glycoprotein present in the complex. Appropriate conditions for effecting such contact are well known to those skilled in the art, e.g. the complex may be redissolved in a suitable buffer and the antibody, dissolv d in the sam or a compatable buffer add d at a t mperatur , e.g. 37°C, at which activity of th antibody is retained.

The antibody used in this method may be either a polyclonal or a monoclonal antibody which is detectable e.g. because an identifiable label such as a radiolabel or a fluorescent label has been attached to it. Alternatively, the antibody may be detected by use of a second antibody directed it, the second antibody being labeled or having an enzyme substrate bound to it.

For example, if the glycoprotein to be detected is desialylated hCG the antibody may be a serum or monoclonal antibody directed to a determinant on the subunit such as an antibody directed to the carboxy terminal region of the subunit. In the experiments which follow asialo hCG, a type of desialylated hCG, has been detected. It is to be understood that desialylated is intended to encompass both glycoproteins which do not naturally include sialo groups and these which naturally include sialo groups, but from which they have been removed. The latter situation is believed to be a general indicator of disease and has been so implicated in choriocarcinoma and hydatiform mole.

The presence of antibody bound to the lectin-glycoprotein complex may be readily detected using well known techniques. Thus, if the antibody is fluorescently labeled with a moiety such as a fluorescent dye covalently bound to the antibody the fluorescent emission of the dye upon excitation with appropriate electromagnetic radiation such as ultraviolet radiation may be measured or detected using a c nventional fluorimeter. In a similar mann r a radi activ isotope such as $I^{125}$ bound to the antibody may be detected using a coventional scintillation spectrometer.

By comparing the results obtained using such methods, e.g. the amount of radioactivity, with those obtained using a control sample one may determine that the desialylated glycoprotein is present in the biological fluid.

A method for quantitatively determining the amount of a soluble desialylated glycoprotein in a sample of a biological fluid comprises contacting the biological fluid with a suitable amount of an appropriate lectin capable of selectively binding to the desialylated glycoprotein to produce a complex. The resulting complex is separately recovered from the biological fluid. The complex so recovered is contacted under appropriate conditions with a predetermined amount of at least one detectable antibody directed to an antigenic determinant on the desialylated glycoprotein and capable of selectively binding to glycoprotein present in the complex. The amount of antibody so bound is determined and, thereby, the amount of desialylated glycoprotein in the biological fluid determined. In one embodiment, the desialylated glycoprotein to be quantitatively determined is hCG.

One application of the methodology for quantitatively determining soluble desialylated glycoprotein involves the diagnosis of a disease associated elevated levels of desialylated hCG such as choriocarcinoma or hydatiform mole. By quantitatively determining the amount of desialylated hCG in a biological fluid from a patient and comparing this amount with the amount d termined for a n rmal pati nt, dis ases such as choriocarcinoma or hydatiform mole may be diagnosed.

Alternatively, a method for determining the presence of soluble desialylated glycoprotein in a biological fluid comprises contacting a sample of the biological fluid with at least one antibody directed to an antigenic determinant on the desialylated glycoprotein and capable of binding to the glycoprotein to produce a complex. The resulting complex is separately recovered from the biological fluid. The so recovered complex is contacted with a suitable amount of an appropriate detectable lectin. The presence of the lectin so bound is detected and, thereby, the presence of desialylated glycoprotein in the biological fluid is determined. The detectable lectin is radiolabeled or fluorescently labeled.

In the same manner as described previously for the method in which the sample is contacted first with a lectin and then with a detectable antibody, the preceding method may be rendered quantitative as may the various related methods which follow.

The invention also provides a method for determining the presence of a soluble desialylated glycoprotein in a biological fluid which comprises contacting a sample of the biological fluid with a suitable amount of an appropriate detectable lectin capable of selectively binding to the desialylated glycoprotein to produce a complex. The complex, under appropriate conditions, is contacted with at least one antibody directed to an antigenic determinant on the desialylated glycoprotein and capable of selectively binding to glycoprotein in the complex. The resulting compl x is separately recover d from th biological fluid. Th pr sence of l ctin bound t the r covered compl x is detect d and, th r by, the presence of desialylat d glycoprotein in th biological fluid is d termined.

The preceding method may be carried out in a different sequence. Thus a method for determining the presence of soluble desialylated glycoprotein in a biological fluid comprises contacting a sample of the biological fluid with at least one detectable antibody directed to an antigenic determinant on the desialylated glycoprotein and capable of binding to the glycoprotein to produce a complex. The complex is contacted with a suitable amount of an appropriate lectin capable of selectively binding to glycoprotein present in the complex. The resulting complex is separately recovered from the biological fluid. The presence of antibody bound to the recovered complex is detected and, thereby, the presence of desialylated glycoprotein in the biological fluid is determined.

In another embodiment, the invention provdies a method for determining the presence of a soluble desialylated glycoprotein in a biological fluid which comprises subtantially concurrently contacting a sample of the biological fluid under appropriate conditions with both a suitable amount of an appropriate lectin capable of selectively binding to the desialylated glycoprotein to produce a recoverable complex and at least one detectable antibody directed to an antigenic determinant on the desialylated glycoprotein capable of selectively binding to the glycoprotein as well as to the recoverable complex. The resulting complex is separately recovered from the biological fluid. The presence of antibody bound to the recovered complex is det cted and, th reby, the presenc of desialylated glycoprotein in th bi logical fluid is det rmin d.

The preceding method may be varied by substituting a detectable lectin for the detectable antibody. Thus, a method for determining the presence of soluble desialylated glycoprotein in a biological fluid comprises contacting a sample of the biological fluid under appropriate conditions with both an antibody directed to an antigenic determinant on the desialylated glycoprotein to produce a recoverable complex and a suitable amount of an appropriate detectable lectin capable of selectively binding to the desialylated glycoprotein as well as to the recoverable complex. The resulting complex is separately recovered from the biological fluid. The presence of lectin bound to the recovered complex is detected and, thereby, the presence of desialylated glycoprotein in the biological fluid is determined.

## Experimental Details

### Reagents:

HCG, (CR121; biopotency, 13,450 IU/mg Second International Standard), hCG β (CR123), β-CTP (hCG 123-145) and their asialo forms (ashCG β, asβ-CTP, ashCGβ 123-145) were prepared as described earlier (13-15). HLH (hLH-I-1 AFP-4345B; biopotency, 6000 IU/mg WHO International Standard of urinary FSH/LH70) was provided by the National Pituitary Agency of NIAMDD. Antisera to β-CTP (R525 and R529) and as β-CTP (R141) were characterized previously (8,16).

The characteristics of the monoclonal antibodies against hCG β (B101, B105 and B107) have been described elsewhere (17). Monoclonal antibody B101 has an equilibrium association constant (Ka) of 7 x 108 M-1 for hCG, and cross-reactivities of 9 and 2% for hCGβ and hLH, respectively. The Ka of B105 for hCG is 1.5 x 1011 M$^{-1}$ and it cross-reacts 100% with both hCG β and hLH. The Ka of B107 for hCG is 4 x $10^{10}$ M$^{-1}$ and it has only 0.1% cross-reactivity with hCG β and less than 0.5% cross-reactivity with hLH.

Monoclonal antibody, B101, was conjugated to CNBr activated Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ) according to the manufacturer's instructions. Iodinated ashCG, hCG and antibodies were prepared using Na$^{125}$-I (Amersham Corp., Arlington Heights, IL) with Iodogen (Pierce Chemcial Co., Rockford, IL) as an oxidizing agent as described by Fraker and Speck (18). Peanut l ctin, *Arachis hypogea* agglutinin, (PNA) and castor bean lectin, *Ricinus communis* agglutinin, (RCA) w re coval ntly linked to

Agarose were obtained from E-Y Laboratories Inc. (San Mateo, Ca.).

Clinical specimens:

First morning voided or 24 hours collected urines were obtained from patients with trophoblastic tumors at New England Trophoblastic Disease Center of Brigham and Women's Hospital, Boston, MA and at the Medical College of Wisconsin, Milwaukee, WI. First morning voided or random urines were also collected from normal pregnant women at the Columbia Presbyterian Medical Center, New York, NY.

Filtration of the urine on sephadex G-100:

One hundred ml of urine from a woman with choriocarcinoma and a normal pregnant woman were lyophilized and redissolved in 10 ml or 0.05 M Tris-HCl buffer containing 0.1 M NaCl (pH 7.4). The samples were chromatographed on a column of Sephadex G-100 (2.5 x 196 cm) previously equilibrated with the same buffer.

Radioimmunoassays:

RIAs of urine specimens processed by gel filtration were performed using antisera R529 (14) and R141 (8) to determine total hCG, ßCTP and ashCG CTP immunoreactivities, respectively, as described by Amr et al. (6). An extration step was added to the RIA using R141 as antiserum (R141 RIA) in order to improv its sensitivity and to apply it t the m asurem nt of ashCG in urin specim ns without gel filtration. Four ml of standards containing 0.05-1.0 pmol s ashCG/ml or urine specimens (previously adjusted to pH 7.4 with

NaOH and centrifuged at 3,000 xg for 15 min), were pipetted into 12 x 75 mm polystyrene tubes. Two hundred microliters of 5% suspension of B101 conjugated Sepharose 4B (B101-Sepharose 4B) in assay buffer (buffer A: 0.1% bovine gamma-globulin, pH 7.4) were pipetted into each tube. The tubes were capped, placed horizontally on a Labquake Shaker (Lab Industries, Berkeley, Ca.) and incubated for 2 hours at room temperature with shaking in order to extract hCG from the samples. The tubes were centrifuged for 30 min at 3,000 xg. The supernatants were removed by aspiration and the pellets were washed with 2 ml of 0.01 M PBS (pH 7.4). One ml of 10% formic acid was added and the tubes incubated for 30 min at 3,000 xg. One-half ml aliquots of the supernatants were removed and pipetted into separate 12 x 75 mm polystyrene tubes. The formic acid was evaporated in a Savant Speed Vac Concentrator (Savant Instruments Inc., Hicksville, NY) and the residue was dissolved in 100 microliters of buffer A. The ashCG concentrations were then determined using the R141 RIA.

Lectin-immunoradiometric assays (LIRMA):

The conditions for the LIRMA's were optimized as follows: The amounts of PNA-Agarose and RCA-Agarose and time of incubation at room temperature required to give maximum adsorption of ashCG from buffer A were determined. The amount of iodinated antibody added to the assay was chosen by determining the amount of tracer which gave the highest rati- of specific counts bound ov r non-specific binding (NSB: binding of tracer in absenc of ashCG). Time studies of radiolabeled antibody binding indicated that equilibrium was not attain d until 96 hours of incubation at 4°C. How v r,

since an incubation period of 48 to 72 hours was sufficient to obtain high assay sensitivity, it was the chosen time of incubation.

Prior to assay, urine samples were adjusted to pH 7.4 with NaOH and centrifuged at 3,000 xg for 15 min. Duplicate or triplicate 4 ml aliquots of urine (standards containing appropriate concentration of ashCG in buffer A or buffer A alone for determination of NSB) were pipetted into 12 x 75mm polystyrene tubes. Two-hundred microliters of 10% suspension of PNA-Agarose or RCA-Agarose in buffer A were pipetted into each tube. The tubes were capped, placed horizontally on a Labquake Shaker and incubated for 2 hours at room temperature in order to extract ashCG from the samples. The tubes were centrifuged for 30 min at 3,000 xg. The supernatants were removed by aspiration and the pellets were washed with 2 ml of wash buffer (buffer B: buffer A containing 1% Tween 20). One hundred microliters buffer A containing approximately 50,000 CPM tracer ($^{125}$I-R525, $^{125}$I-B105 or $^{125}$I-B107) were added to each tube. The samples were incubated for 48-72 hours at 4$^{o}$C with shaking on a Bellco Shaker. The tubes were then washed two times with 2 ml of buffer B to reduce NSB. The radioactivity remaining after washing was determined in a Packard Auto-Gamma Scintillation Spectrometer. Data reduction for the generation of standard curves and ashCG concentrations of urine specimens was accomplished using a four parameter logistic fit (19).

Immunoradimetric assay (IRMA):

Th IRMA f hCG was conducted using B101-Sepharose 4B to xtract hCG and radiolabeled purifi d antis rum

to β-CTP (R525) to measure urinary hCG as described by Armstrong et al. (20).

Results:

R141 RIA:

Typical standard curves generated for the RIA with and without an extraction step are shown in Figure 1. The ashCG dosages which resulted in binding equivalent to 90% ($ED_{90}$) and 10% ($ED_{10}$) of Bo (binding in the absence of ashCG) were used as the limits of the usable range for the standard curves. The $ED_{90}$ and $ED_{10}$ for the direct method, which does not employ an extraction step, corresponded to 0.8 and 20 pmoles ashCG/ml respectively. In contrast, the $ED_{90}$ and $ED_{10}$ of the RIA utilizing an extraction step were 0.05 and 1.0 pmoles ashCG/ml, respectively. Thus, the use of an extraction step resulted in approximately a 16 fold improvement in assay sensitivity. The cross-reactivities of hCG in the assays with and without extraction step were: 0.69 and 0.15% at $ED_{50}$, 7.20 and 6.15% at $ED_{90}$ and 0.17 and 0.03% at $ED_{10}$, respectively.

Figure 1 also shows the dose response curve of ashCG extracted from a pool of normal male urine for which portions had been augmented with dosages identical to those employed in the standard curve. The slope and $ED_{50}$ of the dose response curve in the extraction method were -1.401 and 0.250 pmoles/ml for ashCG in urine, compared to -1.318 and 0.238 pmoles/ml for ashCG in buffer. These differences were not statistically significant. Intra- and int rassay varianc for th extraction m thod have been stablish d utilizing normal mal urin to which 0.25 pmol s ashCG/ml wer added. At this dosage of ashCG, th co ffici nts of

intra- and interassay variance were 9.72% (n=10) and 17.81% (n=10), respectively.

Lectin-immunoradiometric assays:

Typical standard curves for ashCG binding in the LIRMA's using PNA and RCA as extraction reagents and R525, B105 or B107 as antibodies are shown in Fig. 2 A, B, C, D. The ashCG dosages which gave binding equivalent to 10% ($ED_{10}$) and 90% ($ED_{90}$) of the maximum binding (Bmax) are desginated as the limits of the usable range of the standard curves. $ED_{10}$ and $ED_{90}$ corresponded to 0.01 and 2.5 pmoles ashCG/ml in PNA-$^{125}$I-R525 system, 0.0002 and 0.05 pmoles ashCG/ml in PNA-$^{125}$I-B105 system, 0.005 and 0.6 pmoles ashCG/ml in PNA-$^{125}$I-R525 system. Thus, these assays could give 250, 2500, 120 and 100 fold usable ranges, respectively.

The insert in Fig. 2A shows the dose reponse curves of ashCG extracted with PNA from normal male urine which had been augmented with dosages identical to those used in the standards. The dose response curve for ashCG in urine was essentially identical to that for ashCG in buffer A in the PNA-$^{125}$I-R525 system. The slope and $ED_{50}$ were 1.141 and 0.136 pmoles/ml for ashCG in urine compared to 1.051 and 0.122 pmoles/ml for ashCG in buffer A in PNA-$^{125}$I-R525 system. In contrast, as shown in Fig. 2D, the dose response curve obtained using the RCA-$^{125}$I-R525 system for measurement of ashCG in urine and in buffer A do not coincide. Therefore, assays of clinical specimens were conducted using standards diluted in normal mal urine in order to compensat for the low r r c very of hCG from urine by RCA.

The dose response curves for ashCGβ, asβ-CTP, hCG, hCGβ and hLH added to buffer A are also shown in Fig. 2. The cross-reactivities of these standards in the assay systems are summarized in Table 1. Whereas the PNA-$^{125}$I-R525 system can detect ashCG, ashCGβ and free asβ-CTP, the PNA-$^{125}$I-B105 system can only detect ashCG and ashCGβ and the PNA-$^{125}$I-B107 system detects only dimeric ashCG. Since RCA does not recognize asialo O-serine linked carbohydrate side chains in the β-CTP region, RCA-$^{125}$I-R525 system detects only ashCG and βashCG . The cross-reactivities of hCG, hCG and hLH were low in all the assay systems. Intra- and interassay variance for these LIRMA's have been established utilizing normal male urine containing the dosages of ashCG coinciding approximately with $ED_{50}$. These are also shown in Table 1.

Analyses of gel-filtration urine concentrates:

The gel filtration profiles of the concentrated urine from a choriocarcinoma patient and a normal pregnant woman are shown in Fig. 3. The elution profile of the choriocarcinoma urine concentrate had a major peak of hCG immunoreactivity as determined by an RIA using an antiserum to β-CTP (R529) which coincided with the elution volume (Ve) of $^{125}$I-hCG. A peak of ashCG which eluted under the peak of hCG was detected using the R141 RIA and the PNA-$^{125}$I-R525 and RCA-$^{125}$I-R525 LIRMA's. The proportion of ashCG to toal hCG in this first peak was 17% using PNA-$^{125}$I-R525, 16% using RCA-$^{125}$I-R525 and 16% using the R141 RIA.

A s cond peak ofβ-CTP immun r activity compos d of l w molecular w ight m lecul s was also d tected in the lution profile using the R529 RIA, along with a cor-

Table 1

Range, Crossreactivity and Validation of Each Assay

| | PNA-$^{125}$I-R525 | PNA-$^{125}$I-B105 | PNA-$^{125}$I-B107 | RCA-$^{125}$I-R525 | R141-RIA (Extraction Method) |
|---|---|---|---|---|---|
| Assay Range (pmoles ashCG/ml) | 0.01 - 2.5 | 0.0002 - 0.5 | 0.005 - 0.6 | 0.002 - 0.2 | 0.05 - 1.0 |
| Crossreactivity (%) | | | | | |
| ashCG (reference) | 100 | 100 | 100 | 100 | 100 |
| ashCGβ | 100 | 0.00 | 0.00 | 0.00 | NT |
| asβ-CTP | 100 | 0.00 | 0.00 | 0.00 | NT |
| hCG | 0.1 | 0.06 | 0.00 | 0.03 | 0.7 |
| hCGβ | 0.03 | 0.15 | 0.00 | NT | NT |
| hLH | 0.00 | 0.15 | 0.00 | NT | NT |
| Variance (%) | | | | | |
| Intra-assay | 6.8 | 8.3 | 9.0 | 5.1 | 9.7 |
| Inter-assay | 18.6 | 20.1 | 22.3 | 16.3 | 17.8 |

NT = not tested

responding peak of as$\beta$-CTP immunoreactivity detected by the R141 and PNA-$^{125}$I-R525 LIRMA. However, the RCA-$^{125}$I-R525 system did not detect the ashCG to total hCG in the second peak was 110% in PNA-$^{125}$I-R525 and 115% in R141 RIA. In contrast, the gel filtration elution profile for the normal pregnancy urine concentrate had only a single peak of $\beta$-CTP immunoreactivity eluting in the vicinity of $^{125}$I-hCG as determined by the R529 RIA. Also, no ashCG immunoreactivity was detected within this region using the R141 RIA; whereas, very slight amounts were detected using the PNA-$^{125}$I-R525 LIRMA (0.18% of total hCG) and the RCA-$^{125}$I-R525 LIRMA (0.14% of total hCG). Crossreactivity of hCG in the various assays (Table 1) could account for these small amounts of apparent asialo hCG immunoreactivity.

Assays of clinical spcimens:

The PNA-$^{125}$I-R525 LIRMA, RCA-$^{125}$I-R525 LIRMA and R141 RIA with an extraction step were utilized to measure ashCG in urine specimens from patients with gestational trophoblastic tumors and women during normal pregnancy. The total concentrations of hCG in these specimens were determined utilizing the B101-R525 IRMA (20).

The means of the percentages of asialo hCG concentrations over toal hCG concentrations obtained using the three different methods are shown in Table 2. The Newman-Keuls Multiple Range Test (21) was used to determine if the mean values for the three groups of subjects were significantly different from each other. The m an percentag s of asialo hCG ver total hCG concentration for specim ns from choriocarcinoma patients obtain d using all three assays were significantly different from those for hydatidif rm

Table 2

Mean Percentages of Asialo hCG Over Total hCG Concentrations ± Standard Deviations in Specimens from Women with Gestational Trophoblastic Tumors and Normal Pregnant Women

| Method | Pregnancy | Hydatidiform Mole | Choriocarcinoma |
|---|---|---|---|
| R141 RIA (Extraction Method) | 0.937 ± 1.276 (n = 11) | 3.384 ± 3.739* (n = 11) | 11.765 ± 8.76** (n = 6) |
| PNA-R525 LIRMA | 0.049 ± 0.045 (n = 6) | 2.693 ± 3.542* (n = 10) | 13.726 ± 7.350** (n = 6) |
| RCA-R525 LIRMA | 0.178 ± 0.304 (n = 10) | 0.504 ± 1.000* (n = 9) | 2.044 ± 0.709** (n = 5) |

* Not significantly different (p †.05) from normal pregnancy

**Significantly different (p $.05) from normal pregnancy and hydatidiform mole

mole and normal pregnancy. However, the values for hydatidiform mole were not significantly different from normal pregnancy in any of the assays.

Discussion:

Several reports have indicated that the proportion of asialo forms of hCG present in the urine of choriocarcinoma patients are in marked excess relative to the levels in the urine during normal pregnancy (4-7). Although measurements of ashCG might be useful in the detection of trophoblastic tumors, routine assays for ashCG for use in clinical studies have not been developed previously. We have examined the use of new assay systems to measure ashCG in urine specimens and conducted a preliminary investigation of their clinical utility. These methods can be used to obtain highly specific and sensitive measurements of ashCG. They also offer greater ease of performance than the previously used methodology which required gel filtration of specimens prior to RIA (6,7). The cross-reactivities of hCG, hCG$\beta$ and hLH are very low in all of the newly developed systems.

The extreme selectivity of the LIRMA for asialo forms of hCG can be attributed to the carbohydrate specificity at the lectins employed. Peanut lectin, (PNA), has high specificity for the terminal carbohydrate structure Gal$\beta$1$\longrightarrow$3GalNAc (9,10); and castor bean lectin (RCA), is highly specific for the terminal structure of Gal$\beta$1$\rightarrow$4GlcNAc (9). When sialic acid is removed from hCG, its unique $\beta$-CTP region has four structures of O-serine linked Gal$\beta$1$\rightarrow$3GalNAc (2), and each of its and subunits has two structures of N-asparagine linked Gal$\beta$1$\longrightarrow$4GlcNAc (1). Therefore, PNA and RCA r c gniz asialo O-serin and N-asparagine link d carbohydrate side chains, respectively.

As summarized in Table 3, the LIRMAs have the ability to distinguish between various forms of asialo hCG based on the specificities of the lectins and antibodies employed. The PNA-$^{125}$I-R525 system detects ashCG, ashCG $\beta$ and free as$\beta$CTP, indicating simultaneous binding of the lectin and antibody to the $\beta$CTP region. This finding was unexpected considering the potential for steric hindrance of binding two large molecules to this relatively small peptide. The PNA-$^{125}$I-B105 system, which uses a monoclonal antibody to hCG$\beta$, detects ashCG and ashCG$\beta$. Since B107 recognizes only dimeric hCG, PNA-$^{125}$I-B107 detects only ashCG. PNA recognizes asialo O-serine linked carbohydrate moieties which are localized in $\beta$-CTP region (2,10) and RCA recognizes N-asparagine linked carbohydrate moieties which are in $\alpha$ and $\beta$ subunits (1). This difference in demonstrated by the inability of the RCA-$^{125}$I-R525 LIRMA to detect free as$\beta$-CTP. It should be possible to construct similar LIRMA systems to measure a variety of soluble glycoproteins by employing lectins and antibodies with different carbohydrate and peptide binding specificities.

Amr et al. have reported the presence of two different molecules with as$\beta$CTP immunoreactivity in choriocarcinoma urine using the R141 RIA (6). The larger form had a G-100 elution volume similar to that of native hCG whereas the smaller form had an elution volume similar to that of to free asialo $\beta$CTP. These desialylated forms of hCG were not detectable in serum from the same patients.

The pr s nt study confirms the findings of Amr et al. (6) using improved method l gi s which can be appli d t the routine det ction of th se asialo forms of hCG

Table 3

Characteristics of Lectin-Immunoradiometric Assays for Desialylated Forms of hCG

| Method* | Agarose Linked Lectin | Desialylated Molecules Detected** | Radiolabeled Antibody |
|---|---|---|---|
| A | PNA | ashCG, ashCG$\beta$, ashCG$\beta$-CTP | $^{125}$I-R525 |
| B | PNA | ashCG, ashCG$\beta$ | $^{125}$I-B105 |
| C | PNA | ashCG | $^{125}$I-B107 |
| D | RCA | ashCG, ashCG$\beta$ | $^{125}$I-R525 |

* Dose response curves for methods A-D are shown in Figure 2 in panels A-D, respectively.

**Methods A-C and method D detect molecules with desialylated O-serine linked and desialylated asparagine linked oligosaccharides, respectively.

in clinical specimens. Two peaks of immunoreactivity were present in the Sephadex G-100 elution profile of urine from a patient with choriocarcinoma. Analyses of the first peak with the R141 RIA, PNA-R525 LIRMA indicated that approximately 16% of total hCG was asialo. In contrast, all of the hCG βCTP immunoreactivity in the second peak could be accounted for by the presence of as CTP reactivity using the R141 RIA and PNA R525 LIRMA. Since this smaller molecule with CTP immunoreactivity was not detectable using the RCA-R525-LIRMA, it seems likely that it contains only asialo O-serine linked carbohydrate side chains. This finding would also imply that ashCG but not hCG is proteolysed to release a small peptide from the βCTP region.

Analysis of the gel filtration profile of a urine concentrate from a normal pregnant woman using these assays indicated the presence of only small amounts of ashCG reactivity in the area of the elution volume of hCG. Furthermore, the small molecule with asβCTP reactivity present in the urine from a choriocarcinoma patient was not detected. These findings support the proposal of Amr et al. (6) that the molecule with characteristics similar to those of free asβCTP may be a specific marker for choriocarcinoma.

The PNA-R525 and RCA-R525 LIRMA's and the R141 RIA with an extraction step have been utilized in conjunction with an IRMA for hCG to determine the concentrations of asialo forms of hCG relative to those of total hCG in thr diff r nt subj ct groups (Table 2). The mean percentages of asialo hCG over hCG obtained using all thr e assays were significantly high r for urines from ch ri carcinoma patients than those hydatidiform mol

patients and normal pregnant women. Although the mean values for hydatidiform mole patients were also higher than those for normal pregnant women, the differences were not statistically significant. This appears to be due to the high variance within subject groups. Future studies with serially collected specimens from individuals within each subject group will be conducted in order to obtain a better understanding of the utility of asialo hCG measurements in discriminating between gestational trophoblastic disease and normal pregnancy.

It is interesting to compare the values obtained for asialo hCG in patients with trophoblastic diseases using the different methods. The values obtained using the R141 RIA and PNA-R525 LIRMA. Since the first two methods detect terminal Galβ1→3GalNAc residues and the last method detects terminal Galβ1→4GlcNAc residues, this observation may reflect a greater degree of desialylation of the O-serine linked side chains than the N-asparagine linked side chains in the hCG excreted by these patients. Further studies of the nature of hCG produced by gestational trophoblastic disease patients will be required to answer this question.

The origin of the asialo forms of hCG excreted by trophoblastic disease patients is also not understood. They may be the result of an altered sialylation mechanism in trophoblastic tumor tissue or peripheral desialylation. The improved methods for asialo hCG detection described in this present study should be useful in examining the mechanisms r lat d to the excretion f these unusual forms of hCG.

References

1. Kessler MJ, Reddy MS, Shah RH, Bahl OP, Structures of N-glycosidic carbohydrate units of human chorionic gonadotropin. J Biol Chem 1979; 254: 7901-7908.

2. Kessler MJ, Mise T, Ghai RD, Bahl OP, Structure and location of the O-glycosidic carbohydrate units of human chorionic gonadotropin. J Biol Chem 1979; 254: 7909-7914.

3. Birken S, Canfield RE, Isolation and amino acid sequence of COOH-terminal fragements from the beta-subunit of human choriogonadotropin. J Biol Chem 1977; 252: 5386-5392.

4. Nishimura R, Endo Y, Tanabe Y, Ashitaka Y, Tojo S, The biochemical properties of urinary human chorionic gonadotropin from the patients with trophoblastic diseases. J Endocrinol Invest 1981; 4:349-358.

5. Mizucchi T, Nishimura R, Derappe C, Taniguchi T, Hamamoto T, Machizuki M, Kobata A, Structur s of th sugar chains of human chorionic g nadotropin produced in choriocarcinoma. J Biol Ch m 1983; 258: 14126-14129.

6. Amr S, Wehmann RE, Birken S, Canfield RE, Nisula BC, Characterization of a carboxtyterminal peptide fragment of the human chorionic gonadotropin beta-subunit excreted in the urine of a woman with choriocarcinoma. J Clin Invest 1983; 71:329-339.

7. Amr S, Rosa C, Wehmann R, Birken S, Nisula B, Unusual molecular forms of hCG in gestational trophoblastic neoplasia. Ann Endocrino Paris: 1984; 45:321-326.

8. Birken S, Canfield, RE, Lauer R, Agosto G, Gabel M, Immunochemical determinants unique to human chorionic gonadotropin: Importance of sialic acid for antisera generated to human chorionic gonadotropin β-subunit COOH-terminal peptide. Endocrinology 1980; 106: 1659-1664.

9. Goldstein IJ, Hayes CE, Lectins: Carbohydrate binding proteins. Adv Carbohydr Chem Biochem 1978; 35:128-340.

10. C l LA, Birk n S, Sutphen S, Hussa RO, Pattill RA, Absence of the COOH-terminal peptide on ectopic human chori nic gonad tropin b ta-subunit (hCGbeta). Endocrinology 1982; 110:2198-2200.

11. Yamamoto K, Tsuji T, Tarutani O, Asawa T, Phosphorylated high mannose type and hybrid-type oligosacchoride chains of human thyroglobulin isolated from malignant thyroid tissue, Biochemica and Biophysica ACTA 1985; 938: 84-91.

12. Del Dillano B, Brennan S, Brock P, Bucher C, Liu V, McClure M, Rake B, Space S, Westrick B, Shoemaker H, Zwawski B, Radioimmunometric assay for a monoclonal antibody-defined tumor marker, CA19-9. Clin. Chem. 1983; 29:549-552.

13. Canfield RE, Morgan FJ, Human chorionic gonadotropin. I. Purification and biochemical characterization. In: Berson SA, Yalow RS, eds: Methods in Investigative and Diagnostic Endocrinology. III. Non-pituitary hormones. Amsterdam: North Holland Publishing Co. 1973; 727-733.

14. Morgan FJ, Canfield RE, Vaitukaitis JL, Ross GT, Preparation and characterization of the subunits f hCG. In: B rson SA, Yalow RS, eds. M thods in Investigative and Diagnostic Endocrinology. III. Non-pituitary hormones. Amst rdam: N rth Holland Publishing Co. 1973; 733-742.

15. Morgan FJ, Birken S, Canfield RE, The amino acid sequence of human chorionic gonadotropin: the alpha subunit and beta subunit. J Biol Chem 1975; 250: 5247-5258.

16. Birken S, Canfield R, Agosto G, Lewis J, Preparation and characterization of an improved alpha-COOH-terminal immunogen for generation of specific and sensitive antisera to human chorionic gonadotropin. Endocrinology 1982; 110: 1555-1563.

17. Ehrlich PH, Moustafa ZA, Krichevsky A, Birken S, Armstrong EG, Canfield RE, Characterization and relative oreintation of epitopes for monoclonal antibodies and antisera to human chorionic gonadotropin. Amer J Repord Immunol Microbiol (in press).

18. Fraker PJ, Speck Jr, JC, Protein and cell membrane iodinations with a sparingly soluble chloromide, 1,3,4,6-tetrachloro-3a, 6a-diphenyl-glycouracil. Biochem Biophys Res Comm 1972; 80: 849-857.

19. Robard D, Statistical quality control and routin data proc ssing for radioimmunoassays and immunoradiometric assays. Clin Chem 1974; 20:1255-1270.

20. Armstrong EG, Ehrlich PH, Birken S, Schlatt rer JP, Siris E, Hembree W, Canfield RE, Use of a highly sensitive and specific immunoradiometric assay for detection of human chorionic gonadotropin in urine of normal, non-pregnant and pregnant individuals. J Clin Endo Metab 1984; 59:867-874.

21. Comparisons among treatment means in an analysis of variance. Agricultural Research Service of the U.S. Department of Agriculture, ARS/H/6.

What is claim d is:

1. A method for determining the presence of a soluble desialylated glycoprotein in a biological fluid which comprises:

a) contacting a sample of the biological fluid with a suitable amount of an appropriate lectin capable of selectively binding to the desialylated glycoprotein to produce a complex;

b) separately recovering the resulting complex from the biological fluid;

c) contacting the complex so recovered under appropriate conditions with at least one detectable antibody directed to an antigenic determinant on the desialylated glycoprotein and capable of selectively binding to glycoprotein present in the complex;

d) detecting the presence of antibody so bound; and

e) thereby determining the presence of desialylated glycoprotein in the biological fluid.

2. A method of claim 1, wherein the desialylated glycoprotein is hCG, thyroglobulin, carcinoembryonic antigen or CA19-9.

3. A method of claim 3, wherein the d sialylat d glycoprot in is desialylat d hCG.

4. A method acc rding to claim 3, wher in the antigenic determinant is on the β subunit of desialylated hCG.

5. A method according to claim 3, wherein the antigenic determinant is the carboxy-terminal region of the β subunit of desialylated hCG.

6. A method of claim 1, wherein the biological fluid is urine, blood, semen, salivia or pus.

7. A method of claim 4, wherein the biological fluid is urine.

8. The method of claim 1, wherein the lectin specifically binds to the carbohydrate structure Galβ1→3GalNAc.

9. A method according to claim 8, wherein the lectin comprises peanut lectin derived from Arachis hypogea.

10. The method of claim 1, wherein the lectin specifically binds to the carbohydrate structure Galβ1→4GlcNAc.

11. The method of claim 10 wherein the lectin is a castor bean lectin derived from Ricinus communis.

12. A method according to claim 11, wherein the detectable antibody is radiolabeled or fluorescently labeled.

13. A m th d according to claim 1, wh rein th antibody is a monoclonal antibody.

14. A method for quantitativ ly determining the amount of a soluble desialylated glycoprotein in a biological fluid which comprises:

a) contacting a sample of the biological fluid with a suitable amount of an appropriate lectin capable of selectively binding to the desialylated glycoprotein to produce a complex;

b) separately recovering the resulting complex from the biological fluid;

c) contacting the complex so recovered under appropriate conditions with a predetermined amount of at least one detectable antibody directed to an antigenic determinant on the desialylated glycoprotein and capable of selectively binding to glycoprotein present in the complex;

d) determining the amount of antibody so bound; and

e) thereby determining the amount of desialylated glycoprotein in the biological fluid.

15. A method of claim 14, wherein the desialylated glycoprotein is hCG.

16. A method of diagnosing in a patient a disease such as choriocarcinoma or hydatidiform mole which is associated with the pr sence of elevated lev ls of desialylated hCG which compris s quantitatively

det rmining the amount of d sialylated hCG in a sample of biol.gical fluid from the patient using the method of claim 15, comparing the amount so determined with the amount present in a normal patient and thereby diagnosing the ~~disease~~.

17. A method for determining the presence of a soluble desialylated glycoprotein in a biological fluid which comprises:

a) contacting a sample of the biological fluid with at least one antibody directed to an antigenic determinant on the desialylated glycoprotein and capable of binding to the glycoprotein to produce a complex;

b) separately recovering the resulting complex from the biological fluid;

c) contacting the complex so recovered with a suitable amount of an appropriate detectable lectin;

d) detecting the presence of lectin so bound; and

e) thereby determining the presence of desialylated glycoprotein in the biological fluid.

18. A method of claim 17, wherein the detectable lectin is radiolabeled or fluorescently labeled.

19. A m th d for determining the pres nce of a soluble desialylated glycoprotein in a biological fluid which c mprises:

a) contacting a sample of the biological fluid with a suitable amount of an appropriate detectable lectin capable of selectively binding to the desialylated glycoprotein to produce a complex;

b) contacting the complex under appropriate conditions with at least one antibody directed to an antigenic determinant on the desialylated glycoprotein and capable of selectively binding to glycoprotein in the complex;

c) separately recovering the resulting complex from the biological fluid;

d) detecting the presence of lectin bound to the recovered complex; and

e) thereby determining the presence of desialylated glycoprotein in the biological fluid.

20. A method for determining the presence of soluble desialylated glycoprotein in a biological fluid which comprises:

a) contacting a sample of the biological fluid with at least one detectable antibody directed to an antigenic determinant on the desialylated glycoprotein and capable of binding to the glycoprotein to produce a complex;

b) contacting th complex with a suitabl amount of an appropriate lectin capable of selectively binding to glycoprotein present in the complex;

c) separately recovering the resulting complex from the biological fluid;

d) detecting the presence of antibody bound to the recovered complex; and

e) thereby determining the presence of desialylated glycoprotein in the biological fluid.

21. A method for determining the presence of soluble desialylated glycoprotein in a biological fluid which comprises:

a) substantially concurrently contacting a sample of the biological fluid under appropriate conditions with both a suitable amount of an appropriate lectin capable of selectively binding to the desialylated glycoprotein to produce a recoverable complex and at least one detectable antibody directed to antigenic determinant on the desialylated glycoprotein capable of selectively binding to the glycoprotein and to the recoverable complex;

b) separately recovering the resulting complex from the biological fluid;

c) d tecting the pr sence of antibody so bound to the recovered complex; and

d) thereby determining the presence of desialylated glycoprotein in the biological fluid.

22. A method for determining the presence of soluble desialylated glycoprotein in a biological fluid which comprises:

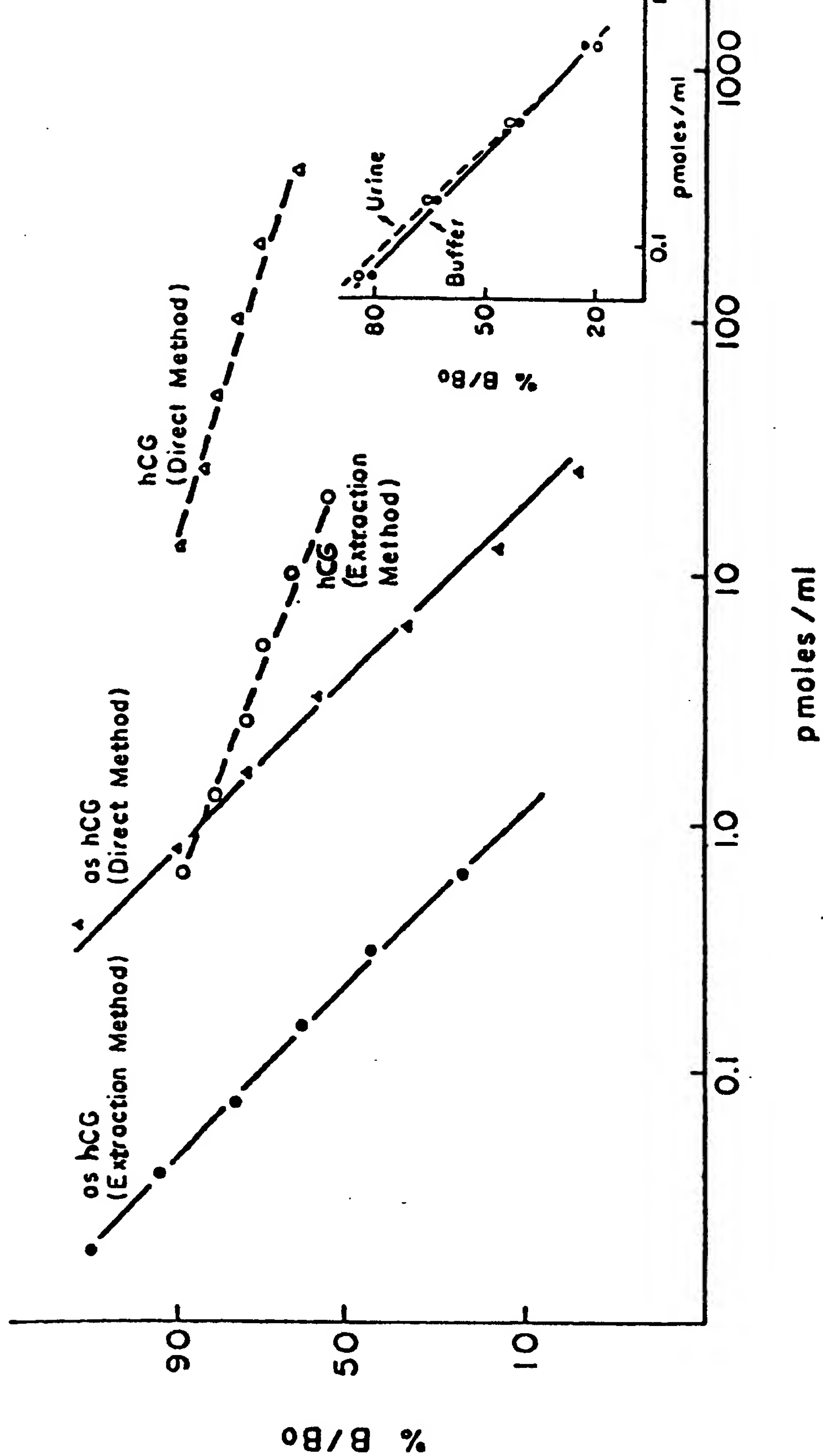
a) substantially concurrently contacting a sample of the biological fluid under appropriate conditions with both an antibody directed to an antigenic determinant on the desialylated glycoprotein to produce a recoverable complex and a suitable amount of an appropriate detectable lectin capable of selectively binding to desialylated glycoprotein and to the recoverable complex;

b) separately recovering the resulting complex from the biological fluid;

c) detecting the presence of lectin bound to the recovered complex; and

d) thereby determining the presence of desialylated glycoprotein in the biological fluid.

FIGURE 1


as hCG
(Extraction Method)
as hCG
(Direct Method)
hCG
(Direct Method)
hCG
(Extraction
Method)
Urine
Buffer
% B/Bo
90
50
10
80
50
20
0.1
1.0
10
100
1000
pmoles/ml
p moles /ml
% B/Bo

2/7

FIGURE 2A

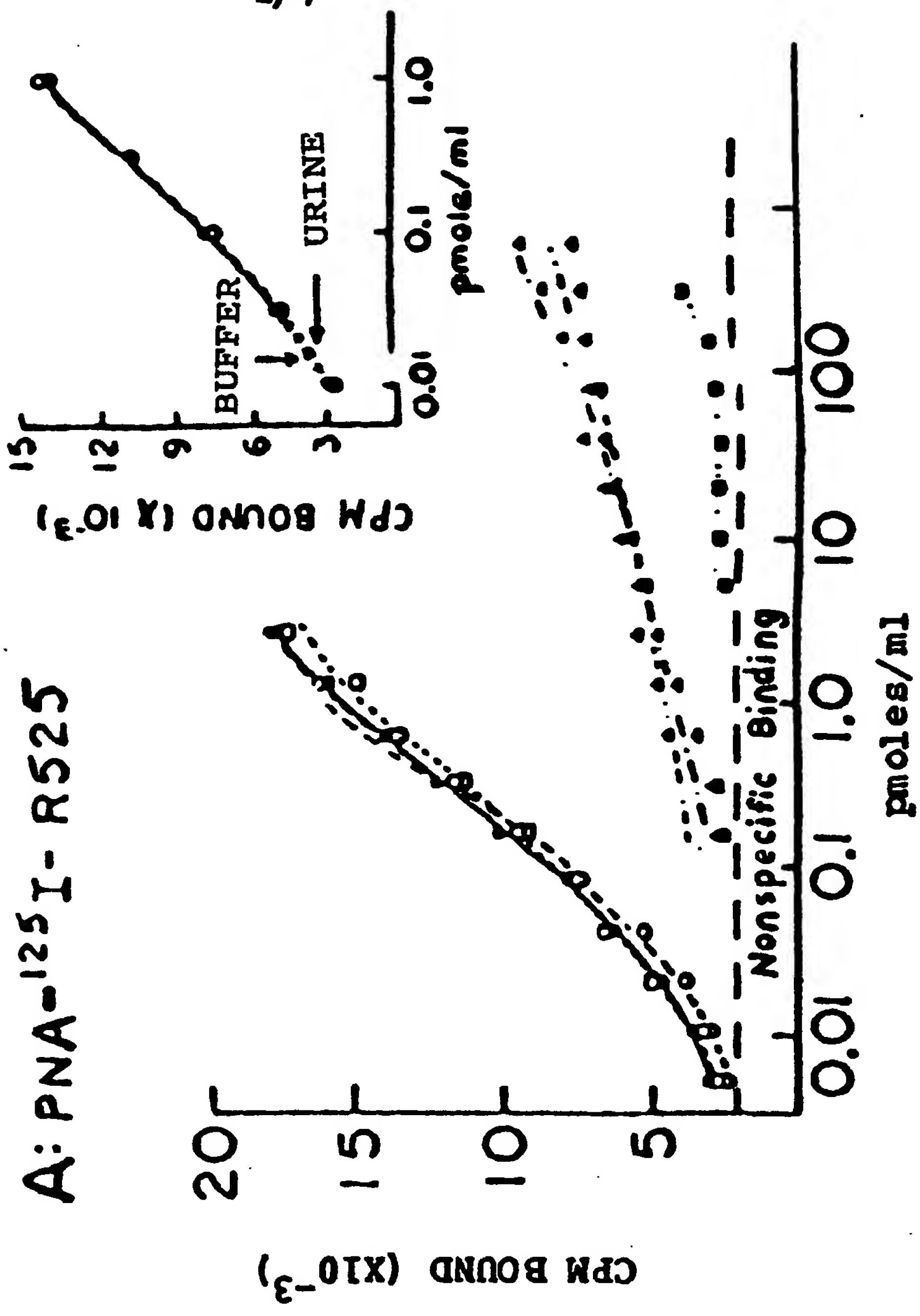
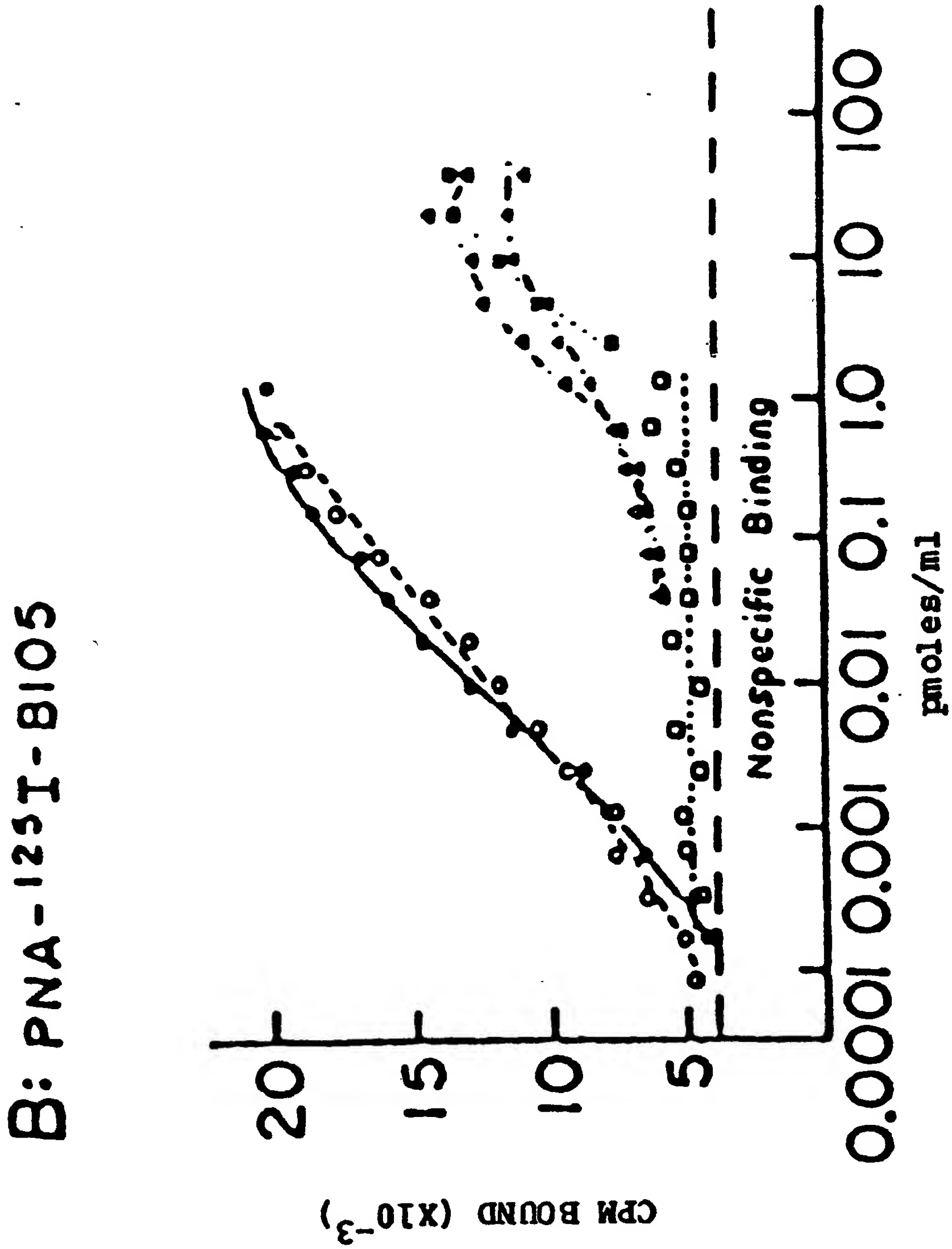

A: PNA-$^{125}$I- R525
CPM BOUND (X10$^{-3}$)
20
15
10
5
0.01
0.1
1.0
10
100
pmoles/ml
Nonspecific Binding
CPM BOUND (X 10$^{-3}$)
15
12
9
6
3
0.01
0.1
1.0
pmole/ml
BUFFER
URINE

FIGURE 2B


B: PNA-$^{125}$I-B105
CPM BOUND (X10$^{-3}$)
20
15
10
5
0.0001
0.001
0.01
0.1
1.0
10
100
pmoles/ml
NonSpecific Binding

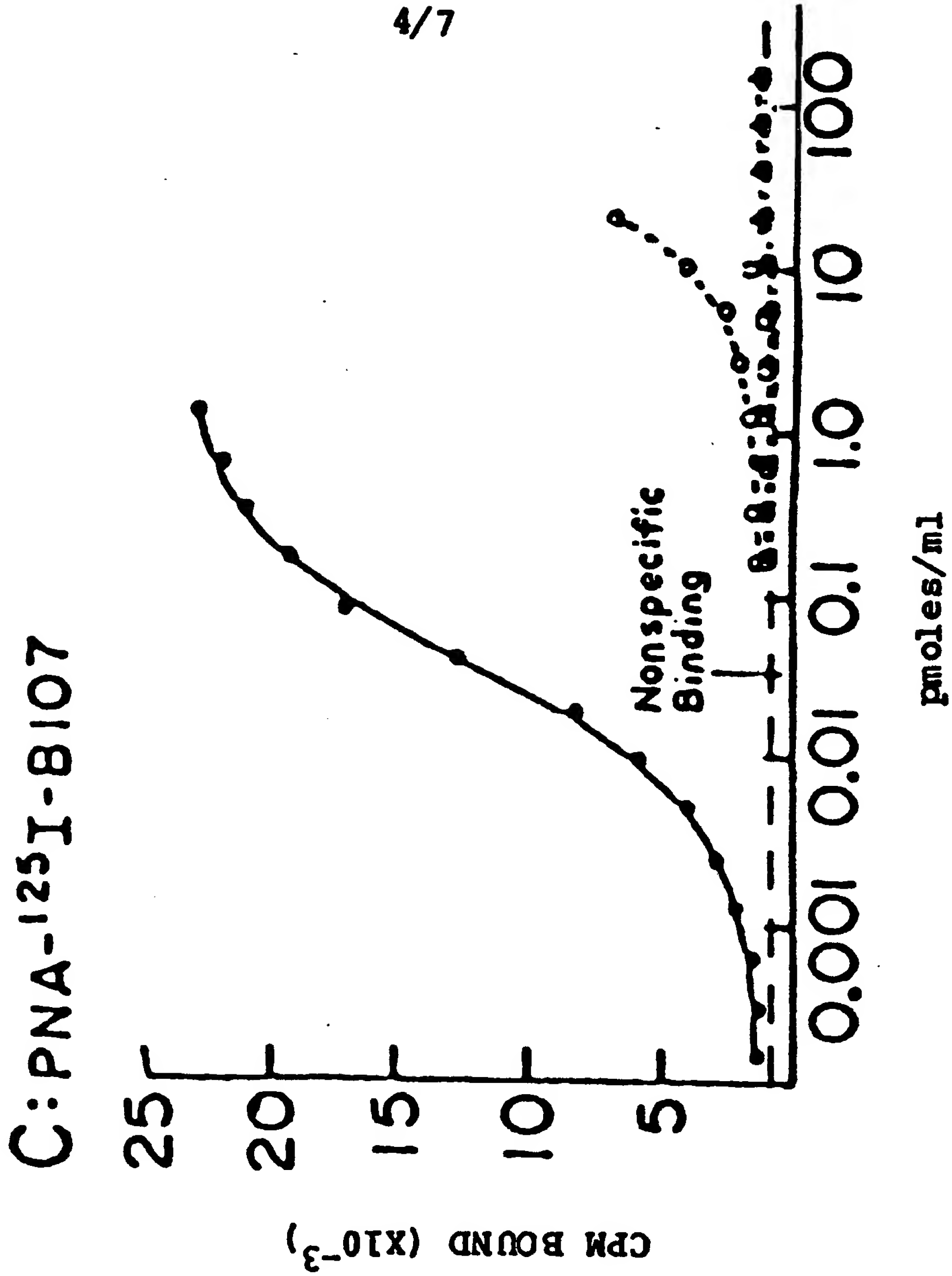

C: PNA-125I-B107
25
20
15
10
5
CPM BOUND (X10^-3)
Nonspecific Binding
0.001
0.001
0.01
0.1
1.0
10
100
pmoles/ml

FIGURE 2C

FIGURE 2D

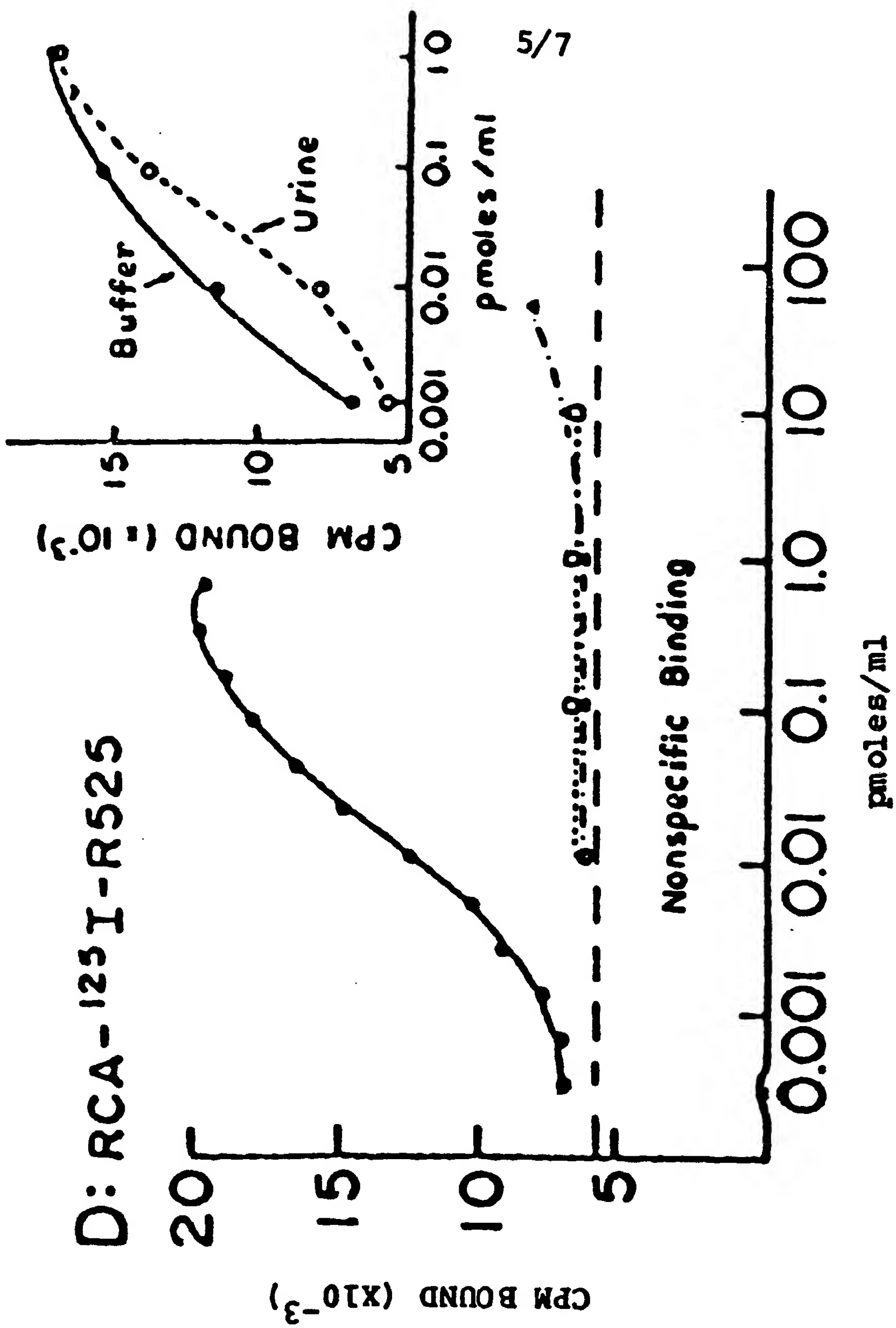

D: RCA-125I-R525
CPM BOUND (X10-3)
20
15
10
5
0.001
0.01
0.1
1.0
10
100
pmoles/ml
Nonspecific Binding
Buffer
Urine
CPM BOUND (x 10-3)
15
10
5
0.001
0.01
0.1
10
pmoles/ml

FIGURE 3A

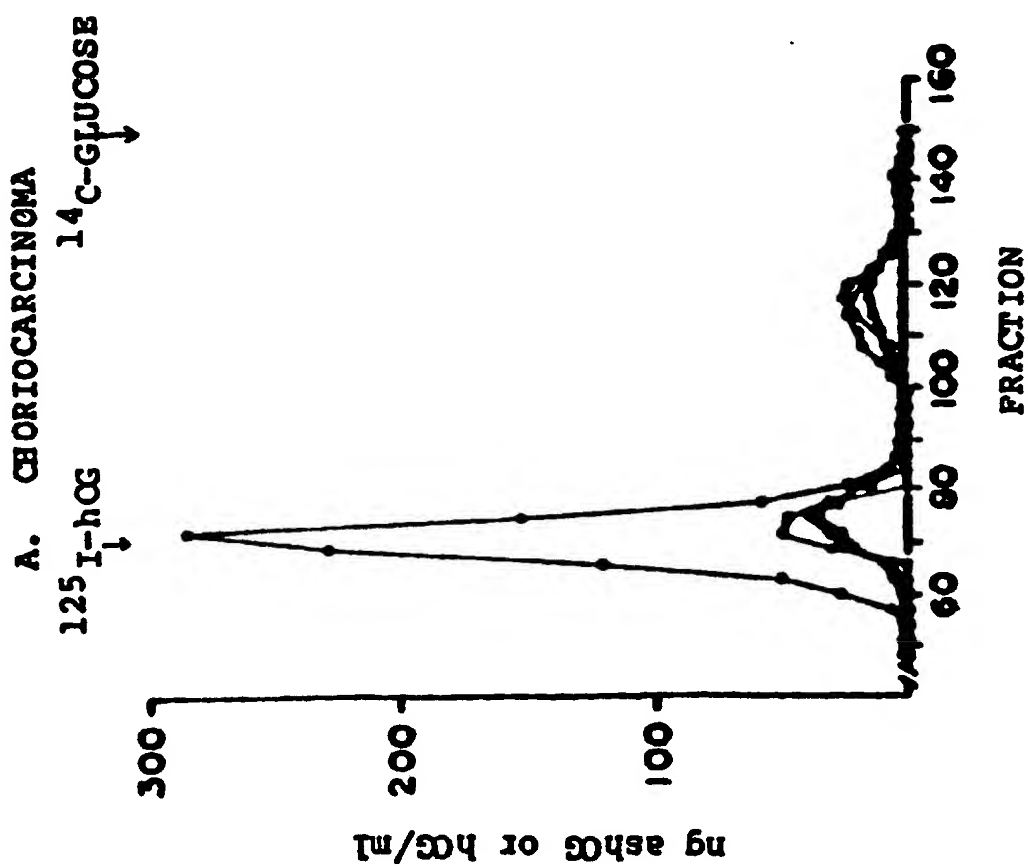
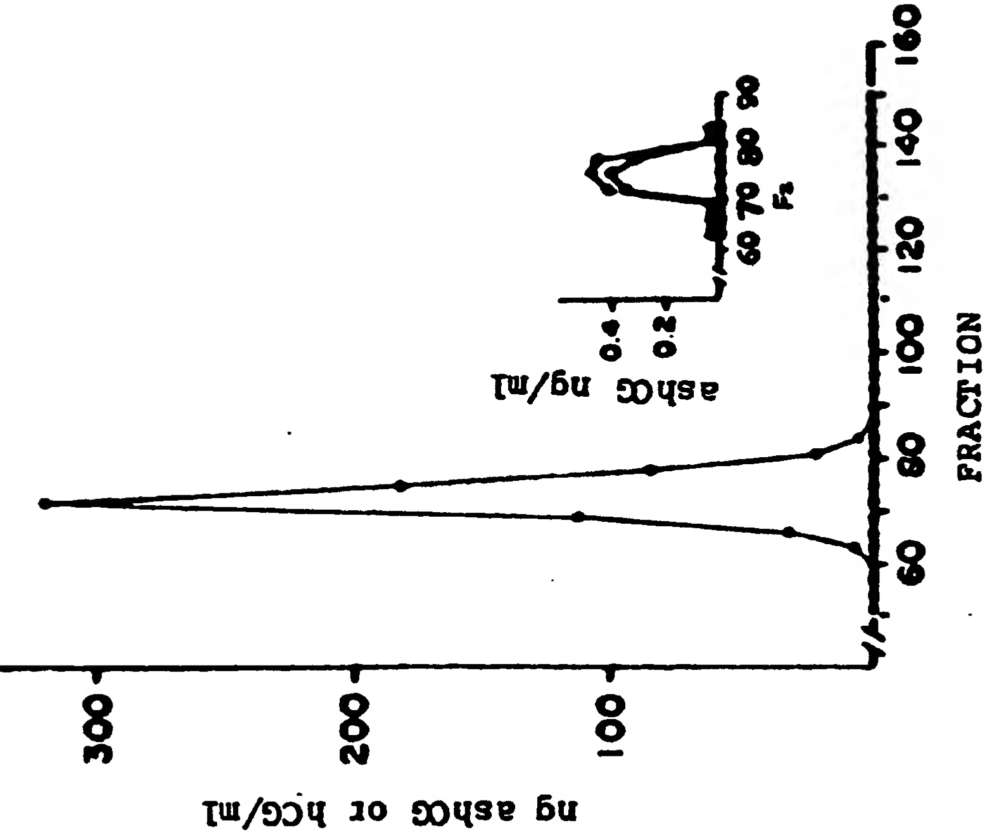

A. CHORIOCARCINOMA
125I-hCG
14C-GLUCOSE
300
200
100
ng ashCG or hCG/ml
60
80
100
120
140
160
FRACTION

FIGURE 3B

B. PREGNANCY



# INTERNATIONAL SEARCH REPORT

International Application No PCT/US86/01399

**I. CLASSIFICATI N OF SUBJECT MATTER** (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

INT. CL.[4] G01N 33/53;33/548;33/566;33/574;33/58

U.S. CL. 436/501,529,548,808,813,818,827

**II. FIELDS SEARCHED**

Minimum Documentation Searched [4]

| Classification System | Classification Symbols |
|---|---|
| U.S. | 436/501,529,548,808,813,818,827 |

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

Chemical Abstracts, 1982-June 1985 under "Gonadotropin, chorionic"

**III. DOCUMENTS CONSIDERED TO BE RELEVANT** [14]

| Category * | Citation of Document, [16] with indication, where appropriate, of the relevant passages [17] | Relevant to Claim No. [18] |
|---|---|---|
| A | US,A, 4,334,017 (PLOTKIN) 08 June 1982 see Abstract | |
| A | US,A, 4,389,392 (ADACHI) 21 June 1983 see Abstract | |
| Y | US,A, 4,508,829 (SULITZEANU) 02 April 1985, see abstract; Column 1, lines 14-19 | 1-22 |
| A,P | US,A, 4,526,871 (AVRAMEAS) 02 July 1985 see Abstract | |

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

**IV. CERTIFICATI N**

| Date of the Actual Completion of the International Search [2] | Date of Mailing of this International Search Report [2] |
|---|---|
| 08 September 1986 | 17 SEP 1986 |
| **International Searching Authority [1]** | **Signature of Authorized Officer [20]** |
| ISA/US | Sidney Marantz Sidney Marantz |

| III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET) | | |
|---|---|---|
| Category * | Citation of Document, [16] with indication, where appropriate, of the relevant passages [17] | Relevant to Claim No [18] |
| A,P | US,A, 4,571,382 (ADACHI) 18 February 1986 see Abstract | |
| Y | Journal of Clinical Investigation, Vol. 71, No. 1 , issued January 1983 (New York, New York) S. Amr et al., "Characterization of a Carboxyterminal Peptide Fragment....", pages 329-339, see Abstract; page 337, column 2, lines 12-32 and page 338, column 1, lines 10-37. | 1-22 |
| Y | Chemical Abstracts, Vol. 102, No. 25, issued 24 June 1985 (Columbus, Ohio, USA), T. Utsunomiya, "Binding of human chorionic gonadotropin...", see page 89, column 1, the abstract no. 102:215,362e, Nippon Sanka Fujinka Gakkai Zasshi 1985, 37(4),555-61 (Japan) | 1-22 |